Considered
12/22/04

PATENT
File No.: 00-53

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Debra G. Gilbertson
Serial No.	:	09/695,121
Filed	:	October 23, 2000
For	:	METHOD FOR TREATING FIBROSIS
	Examiner	: Angell, Jon E.
	Art Unit	: 1635
	Docket No.	: 00-53
	Date	: August 18, 2004

Commissioner for Patents
P.O. Box P.O. Box 1450
Alexandria, VA 22313-1450

Declaration of Christopher Clegg Under 37 C.F.R. § 1.132

Sir:

I, Christopher Clegg, do hereby declare as follows:

1. I am currently employed by ZymoGenetics, Inc., the assignee of the above-named patent application, as Research Director, Immunology.

2. I received a Ph.D. in Zoology from the University of Washington in 1984.

3. I have read the Office Action mailed June 1, 2004 in the above-identified patent application ("the Patent Application"), including the rejection under 35 U.S.C. § 102(e). I am providing this Declaration to assist the patent examiner in evaluating the teachings of Ferrara et al., U.S. Patent No. 6,455,283.

4. Claims 1-6, 9, 11-13, 15, and 17-25 of the Patent Application recite an antibody that specifically binds to a dimeric protein consisting of two polypeptide

chains, wherein each of said polypeptide chains consists of a sequence of amino acid residues selected from the group consisting of residues 230-345 of SEQ ID NO:2, residues 231-345 of SEQ ID NO:2, residues 232-345 of SEQ ID NO:2, residues 233-345 of SEQ ID NO:2, residues 234-345 of SEQ ID NO:2, residues 235-345 of SEQ ID NO:2, residues 236-345 of SEQ ID NO:2, residues 237-345 of SEQ ID NO:2, residues 238-345 of SEQ ID NO:2, residues 239-345 of SEQ ID NO:2, and residues 240-345 of SEQ ID NO:2. SEQ ID NO:2 is the sequence of a human protein referred to in the Patent Application as "zvegf3." This protein is now more commonly known as "PDGF-C" and is described, for example, by Li et al. (*Nature Cell Biol.* 2:302-309, 2000). Residues 235 and 345 of PDGF-C are the approximate boundaries of the active growth factor domain of the protein. As is disclosed in the Patent Application at page 5, domain boundaries are somewhat imprecise and can vary by up to ±5 amino acid residues from stated positions.

5. PDGF-C is secreted as an inactive precursor that requires specific proteolytic cleavage for activation. This cleavage separates the growth factor domain from the remainder of the molecule, which comprises an amino-terminal CUB domain and an interdomain region. The structure and proteolytic activation of PDGF-C are described in the Patent Application, for example at pages 4-6. This requirement for proteolytic activation was subsequently confirmed by others, including Li et al. (*ibid.*).

6. In view of PDGF-C's requirement for proteolytic activation, a person of ordinary skill in the art would reasonably predict that the active region (i.e., the growth factor domain) is buried within the full-length, precursor form of the protein and becomes exposed only after cleavage between the interdomain region and the growth factor domain. This prediction is further supported by recently published work, including that of Fang et al., *Arterioscler Thromb Vasc Biol.* 24:1-7, 2004 (published online before print February 5, 2004), who show that full-length PDGF CC is biologically inactive, whereas PDGF CC growth factor domain activates PDGF receptors. Thus, if the full-length protein were injected into an animal for the purpose of raising antibodies, one could not predict, with a reasonable likelihood of success, that an antibody that specifically binds to an epitope within the growth factor domain would be obtained. Even if a small portion of the CUB domain or the interdomain region was removed, the remaining amino-terminal residues would be expected to mask the active region.

7. I therefore conclude that if an animal was immunized with a polypeptide comprising at least about 80% of the PDGF-C ("VEGF-E") sequence

disclosed by Ferrara et al., one skilled in the art would not reasonably expect to obtain an antibody that specifically binds to a dimeric protein as recited in claims 1-6, 9, 11-13, 15, and 17-25 of the Patent Application.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that the making of willfully false statements and the like is punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and may jeopardize the validity of any patent issuing from this patent application.

8/18/04
Date

Christopher Clegg

PATENT
File No.: 98-60D1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Debra G. Gilbertson
Serial No.	:	09/695,121
Filed	:	October 23, 2000
For	:	METHOD FOR TREATING FIBROSIS
	Examiner	: Angell, Jon E.
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Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Declaration of Henry Francis Pelto III Under 37 C.F.R. § 1.132

Sir:

I, Henry Francis Pelto III, do hereby declare as follows:

1. I am currently employed by ZymoGenetics, Inc., the assignee of the above-named patent application, as Research Associate, Protein Biochemistry.

2. I received a Bachelor of Science degree in Biology from Gonzaga University in May 2002.

3. I have read the Office Action mailed June 1, 2004 in the above-identified patent application ("the Patent Application"), including the rejection under 35 U.S.C. § 102(e). I am providing this Declaration to assist the patent examiner in evaluating the teachings of Ferrara et al., U.S. Patent No. 6,455,283.

4. I performed an experiment to test the ability of antisera raised against a full-length zvegf3 protein to recognize different forms of zvegf3 (also known as

PDGF-C). The antisera, designated "E2243", was raised in a rabbit by immunization with a full-length human zvegf3 polypeptide fused to *E. coli* maltose binding protein (MBP) and affinity purified using the fusion protein.

5. The experiment was carried out in a Western blot format in which samples of various zvegf3 proteins were reduced and electrophoresed on a polyacrylamide gel. The proteins used were: recombinant human zvegf3 growth factor domain, recombinant human zvegf3 full-length, and recombinant human zvegf3 full-length fused to MBP, each at concentrations of 13.9, 41.7, and 125 ng/lane; and conditioned media from HaCat cells expressing full-length human zvegf3. The electrophoresed proteins were then transferred to a nitrocellulose membrane, rinsed, and blocked by overnight incubation in buffer containing 2.5% non-fat dry milk. The primary antibody (E2243 antisera) was diluted to 300 ng/ml and added to the nitrocellulose blot, which was then incubated for 1 hour at room temperature with shaking. The blot was then rinsed, secondary antibody (anti-rabbit IgG conjugated to horseradish peroxidase) was added, and the blot was incubated for 1 hour at room temperature with shaking. The blot was then rinsed, developed with commercially available substrates, and exposed to film for 10 seconds.

6. The Western blot showed that the E2243 antisera recognized all samples of full-length zvegf3 (fused and unfused) and the zvegf3 in the conditioned media. The antisera did not recognize any of the samples of isolated zvegf3 growth factor domain.

7. In summary, antisera raised against a full-length recombinant human zvegf3 fusion protein bound to fused and unfused full-length human zvegf3, but did not bind to isolated human zvegf3 growth factor domain, when tested in a Western blot format.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that the making of willfully false statements and the like is punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and may jeopardize the validity of any patent issuing from this patent application.

18 August 2004

Date

Henry Francis Pelto III